of losing it forever. We do not know exactly the genetic materials that will be needed in the future for maintaining and improving out crops, our animals and our industrial microbial processes. We do know that we will need exotic germplasm of many kinds to deal with problems, some of which are not currently known. Anything that jeopardizes our ability to maintain germplasm for future use reduces our future ability to succeed in keeping up with the demands that will be placed on science to solve problems through breeding and genetic engineering. No one yet knows the genetic treasurers to be found in these important gene banks. Nor do I believe that we yet see the full potential of the genetic engineering technologies that will use these precious materials to make the impossible possible. Not only new cultivars, but also whole new crops, are likely to change forever the dietary profile of humans.

Remember that science is only about 250 years old and most of the scientists who have ever worked are still alive today. We are at the start, not the end, of a logarithmic growth curve. I believe that we are only beginning to understand the biology of plants well enough to approach their management and improvement using scientifically based biological, ecological, and genetic principles. We have important work ahead of us. Major progress awaits us. I can think of no better use of our precious time and energy than in the furtherance of our understanding of crops and cropping systems.

CROP BREEDING, GENETICS & CYTOLOGY

Recombination Values for the *Ms6-W1* Chromosome Region in Different Genetic Backgrounds in Soybean

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ABSTRACT

Recombination values in plants are influenced by genetic factors, environmental conditions, and their interactions. In soybean, *Glycine max* (L.) Merr., the cosegregation method to produce hybrid seed depends on the close linkage of the *Ms6*(pollen fertility) locus and the *W1* (flower color) locus. Twenty-four near-isogenic lines, cosegregating at the *Ms6* and *W1* loci, (*Ms6W1 ms6w1*), were developed with the *ms6-w1* donor cytoplasm and the recurrent parent cytoplasms. The objectives were to determine the recombination values between the *Ms6* and *W1* loci from both testcross and F2-family data. The recombination value for the *Ms6* and *W1* loci from the testcross data in the *ms6-w1* donor cytoplasm was 3.14 ± 0.80 and in the recurrent parent cytoplasms was 3.62 ± 0.89. From the F2-family data, the recombination value for the *Ms6* and *W1* loci in the *ms6-w1* donor cytoplasm was 3.06 ± 0.35 and in the recurrent parent cytoplasms was 4.90 ± 0.35. The recombination values for the F2-family data were statistically significantly different ($P = 0.05$) for the *ms6-w1* donor cytoplasm versus the recurrent parent cytoplasms. However, the recombination values are acceptable to continue to use the cosegregation method to produce hybrid soybean seed.

Recombination values vary in plants as do many other phenotypes. Intrinsic and extrinsic factors affecting recombination may be random or directed for specific chromosome regions (Mock, 1972). The discordant results found by different investigators for the same chromosome region may be attributed to genotype × environment interaction, chromosome structure differences between the parents (Stephens, 1950), and which parent was the male or the female parent (Rhoades, 1941; Burt et al., 1991; Williams et al., 1995). Extensive data show significant differences in recombination values obtained from coupling data as opposed to repulsion data, or backcross (testcross) data as opposed to F2 data (Mather, 1951; Butler, 1968). In addition, controls and treatments must be replicated adequately so that chance extreme values will not be attributed to treatments (Butler, 1977).

In soybean, the pollen fertility locus, *Ms6*, is linked to flower color locus, *W1* (Palmer and Skorupska, 1990). Cross-pollinations of genetic types T295 (*ms6 ms6*) × 'Calland', and Calland × T295H (*Ms6 ms6*), and T295 × 'Cutler' and × 'Hark' gave recombination values for *Ms6-W1* between 2.48 ± 0.1 and 3.18 ± 0.1 (Skorupska and Palmer, 1989). Lewers and Palmer (1993), by using three different Linkage Group 8 genetic stocks, reported recombination values for *Ms6-W1* between 3.86 ± 0.69 and 4.78 ± 1.46. All seven genetic combinations were in coupling phase and indicated close linkage of the two loci.

The cosegregation of the *ms6* and *w1* alleles has been used to produce large quantities of hybrid soybean seed (Lewers et al., 1996). The *W1_* seedlings have purple hypocotyls and purple flowers; *w1 w1* seedlings have green hypocotyls and white flowers. The *ms6 ms6* plants are male sterile and female fertile. Approximately 97% of the purple-hypocotyl seedlings, *W1_*, in a family segregating for the *w1* and *ms6* alleles in coupling phase will be fertile plants, *Ms6_*. More than 92% of the green-hypocotyl seedlings are expected to be male-sterile (*ms6*

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Published in Crop Sci. 38:293–296 (1998).

Table 1. Soybean cultivars, breeding lines, and plant introductions used to generate *Ms6 ms6* backcross-derived lines.

Public lines	Private lines		Ancestral lines	Plant introductions
BSR 101	AGO20	Asgrow Seed Co.	A.K. (Harrow)†	China
Century	AX2858	Asgrow Seed Co.	Manchu	PI 91167†
Corsoy 79	A3307†	Asgrow Seed Co.	Mandarin	PI 261474†
Elgin	CX-155	DeKalb Genetics Corp.	Mandarin (Ottawa)	PI 427099†
Hack†	82-165†	Land O'Lakes, Inc.	Richland	
Hardin	82-378†	Land O'Lakes, Inc.		Japan
Hoyt	J-201	Mycogen Seeds		PI 297544†
	G3917	Novartis Seeds, Inc.		PI 370059
	S1346	Novartis Seeds, Inc.		PI 384474
	P422-57	Pioneer Hi-Bred, Int'l.		
	P596-13	Pioneer Hi-Bred, Int'l.		USSR
	P3010-02	Pioneer Hi-Bred, Int'l.		PI 227333†
	Glenn	ProfiSeed, Inc.		PI 416941
				PI 417076

† These lines or plant introductions are white flower (*w1w1*), other lines or plant introductions are purple flower (*W1W1*).

ms6) plants. Either phenotype can be identified and removed at the first trifoliolate stage.

Palmer and Lewers (1998) have developed 68 near-isogenic lines segregating at the *Ms6* locus. These near-isogenic lines were developed from ancestral, public, and private company breeding lines as recurrent parents. Of these lines, 24 are cosegregating *W1 w1 Ms6 ms6* and 10 are *w1 w1 Ms6 ms6* lines. In addition, these 34 lines are available in both the donor cytoplasm (T295H) and the recurrent parent cytoplasms. The availability of these lines, with the adaptation of the cosegregation method to produce large quantities of hybrid seed, will be useful in research on commercialization of hybrid soybean and on recurrent selection (Lewers and Palmer, 1997).

It is important to know if recombination in the *Ms6-W1* chromosome region is affected by intrinsic and (or) extrinsic factors. The recombination values will determine the resources (seed and land), and environment, that are necessary to maximize hybrid seed production per unit of land area. The objective was to determine the recombination values between the *Ms6* and *W1* loci in the *ms6-w1* donor cytoplasm and the recurrent parent cytoplasms. Linkage determinations were made based upon testcrosses and F2-family segregation.

MATERIALS AND METHODS

The 34 recurrent parents used in the male-sterile conversion program are listed in Table 1. Nine of the recurrent parents are high-yielding accessions introduced into the USA, five lines are important ancestors of modern soybean cultivars, seven are important public cultivars, and 13 are private company cultivars or breeding lines. The pedigree of the *ms6-w1* donor cytoplasm is Hawkeye/Lincoln/2/Grant/3/Amsoy 71/4/Bonus/Cutler (Palmer and Skorupska, 1990). The procedure used to backcross *ms6* into the 34 lines is outlined in Table 2.

Data obtained from testcross and F2-family segregations were used to calculate recombination values. Data were summarized across all cytoplasms and subdivided into the *ms6-w1* donor cytoplasm and also combined across all recurrent parent cytoplasms.

In Season 13, the reciprocal cross combinations were evaluated as BC7F2 families. The genotypes of the BC7F1 plants (testcrosses) were determined by the BC7F2 segregation. Assuming no linkage, the expected frequency of BC7F1 genotypes when both male sterility and flower color are cosegregating is 1 *Ms6 Ms6 W1 W1* : 1 *Ms6 Ms6 W1 w1* : 1 *Ms6 ms6 W1 W1* : 1 *Ms6 ms6 W1 w1*. The observed segregation was compared with the expected 1: 1: 1: 1 segregation by χ^2 analysis. χ^2 Values were calculated and were highly significant. Recombination values were obtained by using the computer program Linkage-1 (Suiter et al., 1983), which uses the maximum likelihood method (Allard, 1956).

In season 13, the BC7F2 families were classified into four genotypic groups. Within BC7F2 families, segregating for both male sterility and flower color, 16 fertile plants were single-plant threshed and evaluated as BC7F2 plant-progeny rows the following summer. The observed segregation data are based upon a total of 800 families (16 plants × two cytoplasm classes × 24 lines). (One line, P596-13 was represented by 32 families × two cytoplasm classes.) Six genotypic groups were

Table 2. Schedule to generate *Ms6 ms6* backcross-derived soybean lines.

Season	Location	Year	Procedure
1	Ames, IA	1987	Pollinate donor parent *ms6 ms6 w1 w1* plants with recurrent parent to make F1.
2	Ames, IA	1988	Pollinate F1 with recurrent parent to make BC1F1.
3	Isabella, PR	1988/89	Advance BC1FI to BC1F2.
4	Ames, IA	1989	Pollinate BC1F2 *ms6 ms6* plants with recurrent parent to make BC2F1.
5	Ames, IA	1990	Pollinate BC2F1 with recurrent parent to make BC3F1.
6	Isabella, PR	1990/91	Advance BC3F1 to BC3F2.
7	Ames, IA	1991	Pollinate BC3F2 *ms6 ms6* plants with recurrent parent to make BC4F1.
8	Ames, IA	1992	Pollinate BC4F1 with recurrent parent to make BC5F1.
9	Isabella, PR	1992/93	Advance BC5F1 to BC5F2.
10	Ames, IA	1993	Pollinate BC5F2 with recurrent parent to make BC6F1.
11	Ames, IA	1994	Pollinate BC6F1 with recurrent parent to make BC7F1 with donor parent cytoplasm. Pollinate recurrent parent with BC6F1 to make BC7F1 with recurrent-parent cytoplasm.
12	Isabella, PR	1994/95	Advance BC7F1 to BC7F2.
13	Ames, IA	1995	Identify cosegregating families (*Ms6 W1 ms6 w1*) or segregating families (*Ms6 ms6 w1 w1*) from reciprocal crosses. Single-plant thresh 4 plants per entry from 4 entries for a total of 16 plants per cross combination. All families selected should have phenotype of recurrent parents.
14	Ames, IA	1996	Identify cosegregating families (*Ms6 W1 ms6 w1*) or segregating families (*Ms6 ms6 w1 w1*). Select one family per cross combination for seed increase and release.

observed for flower color and fertility/sterility. Assuming no linkage, the expected frequencies of fertile BC7F2 genotypes are shown in Table 3.

Following Mather (1951), we derived the maximum likelihood estimator for recombination frequency ($\hat{r}$) from this six-class segregation as the value of r that satisfied the following equation:

$$\frac{2n_A}{r} + \frac{(n_B + n_D + n_E)(1 - 2r)}{r(1 - r)} + \frac{2n_C}{r - 1} + \frac{2n_F(2r - 1)}{(1 - 2r + 2r^2)} = 0$$

where n_i = number of genotypes in Class i. The equation was evaluated numerically to obtain that value of r that made the equation most nearly correct for each sample of progeny. The standard error of this estimate was derived as:

$$\text{S.E.} = \left[\frac{4n}{3} + \frac{2n(1 - 2\hat{r} + 2\hat{r}^2)}{r(1 - \hat{r})} + \frac{16n[\hat{r}(\hat{r} - 1)]}{3(1 - 2\hat{r} + 2\hat{r}^2)}\right]^{-1/2}$$

where n = total number of genotypes observed in the sample.

RESULTS

Backcross (Testcross) Transmission

From the 10 combinations segregating only for male sterility (all white flower) in two cytoplasmic classes, 349 testcross progenies were produced. The ratio of 180 families segregating for male sterility: 169 all fertile families, fit the expected 1:1 ratio ($\chi^2 = 0.35$). The expected 1:1 ratio was realized within the two cytoplasmic classes; $\chi^2 = 2.66$ for the *ms6-w1* donor cytoplasm class, and $\chi^2 = 0.72$ for the recurrent parent cytoplasms class. We had equal transmission of the *Ms6* and ms6 gametes.

From the 24 combinations cosegregating for male sterility and flower color in two cytoplasmic classes, 918 testcross progenies were produced. Segregation for flower color and for sterility for the combined cytoplasmic class as well as for the two separate cytoplasmic classes fit the expected 1:1 ratio (data not given). The observed segregation data, compared with the expected 1:1:1:1 ratio for the testcross cosegregation of male sterility and flower color across all cytoplasms, gave a highly significant $\chi^2 = 798.16$ (Table 4). There was an excess of individuals in the purple flower fertile class and in the segregating flower color segregating sterility class, which indicated linkage. An average recombination value of 3.37 ± 0.60 was calculated for the *Ms6-W1* loci in coupling phase (Table 4). Highly significant χ^2 values were observed within each of the two cytoplasmic classes. The recombination value for the *ms6-w1* donor cytoplasm class for the *Ms6* and *W1* loci was 3.14 ± 0.80 and for the recurrent parent cytoplasms class was 3.62 ± 0.89 (Table 4).

Table 3. Expected frequencies, assuming no linkage, of fertile BC7F2 genotypes.

Expected frequency†	Genotype	Class
1/12	*Ms6 Ms6 w1 w1*	A
2/12	*Ms6 ms6 w1 w1*	B
1/12	*Ms6Ms6 W1 W1*	C
2/12	*Ms6 ms6 W1 W1*	D
2/12	*Ms6Ms6 W1 w1*	E
4/12	*Ms6 ms6 W1 w1*	F

† χ^2 Values were calculated for this 1:2:1:2:2:4 segregation and were highly significant.

Table 4. Recombination values for the *ms6-w1* chromosome region of soybean within the *ms6-w1* donor cytoplasm, recurrent parent cytoplasms, and combined across all cytoplasms. Testcross data from the 24 purple-flowered lines used to produce the cosegregating (*Ms6 W1 ms6 w1*) lines for the male-sterile conversion program.

	Cytoplasm classes and number of testcrosses		
Classes	All cytoplasms	*ms6-w1* donor cytoplasm	Recurrent parent cytoplasms
All fertile, all purple flower	451	223	228
Segregating steriles, all purple flower	16	8	8
All fertile, segregating flower color	15	7	8
Segregating steriles, segregating flower color	436	239	197
χ^2 (1:1:1:1)	798.16	418.81	379.03
R ± S.E.	3.37 ± 0.60	3.14 ± 0.80	3.62 ± 0.89

F2 Family Segregation

From the 10 all-white flower combinations segregating for male sterility in two cytoplasmic classes, 320 F2 families were produced. The ratio of families segregating for male sterility: all fertile families, fit the expected 2:1 ratio for the three cytoplasm classes. Within the all-cytoplasms class, $\chi^2 = 2.81$, within the *ms6-w1* donor cytoplasm class, $\chi^2 = 1.63$, and within the recurrent parent cytoplasms class, $\chi^2 = 1.20$.

For the 24 cosegregating combinations for male sterility by two cytoplasmic groups, 800 F2 families were produced. Segregation for flower color and for sterility for the combined cytoplasmic class as well as for the two cytoplasmic classes fit the expected 3:1 ratio (data not given). The observed segregation data, compared with the expected 1:2:1:2:2:4 ratio for the F2-family segregation of male sterility and flower color across all cytoplasms, gave a highly significant $\chi^2 = 1107.18$ (Table 5). There was an excess of individuals in the purple flower fertile class and in the segregating flower color

Table 5. Recombination values for the *ms6-w1* chromosome region of soybean within the *ms6-w1* donor cytoplasm, recurrent-parent cytoplasms, and combined across all cytoplasms. F2-family data from the 24 purple-flowered lines used to produce the cosegregating (*Ms6 W1 ms6 w1*) lines for the male-sterile conversion program.

	Cytoplasm classes and number of F2 families		
Classes	All cytoplasms	*ms6-w1* donor cytoplasm	Recurrent parent cytoplasms
All fertile, all white flower	0	0	0
Segregating steriles, all white flower	16	7	9
All fertile, all purple flower	266	131	135
Segregating steriles, all purple flower	29	10	19
All fertile, segregating flower color	17	7	10
Segregating steriles, segregating flower color	472	245	227
χ^2 (1:2:1:2:2:4)	1107.18	567.99	541.35
R ± S.E.	3.98 ± 0.25	3.06 ± 0.35	4.90 ± 0.35

segregating sterility class, which indicated linkage. An average recombination value of 3.98 ± 0.25 was calculated for the *Ms6* and *W1* loci in coupling phase (Table 5). The recombination value for the *ms6-w1* donor cytoplasm class was 3.06 ± 0.35 and for the recurrent parent cytoplasms class was 4.90 ± 0.35 (Table 5).

DISCUSSION

The range of recombination values for the testcross data, 3.14 ± 0.80 to 3.62 ± 0.89, and for the F2-family data, 3.06 ± 0.35 to 4.90 ± 0.35, are similar to the reported values of 2.48 ± 0.1 to 3.18 ± 0.1 (Skorupska and Palmer, 1989) and 3.86 ± 0.69 to 4.78 ± 1.46 (Lewers and Palmer, 1993).

For the testcross data, the recombinant phenotypes were randomly distributed among the 24 lines and the two cytoplasms. With the F2-family data, statistically significantly lower recombination values were observed for the *ms6-w1* donor cytoplasm versus the recurrent parent cytoplasms. More recombinant phenotypes were recorded for the recurrent parent cytoplasms class and contributed to the 4.90 ± 0.35 recombination value. The recombinant phenotypes seemed to be randomly distributed within the recurrent parent cytoplasm class. However, in the Hardin Recurrent Parent 4 of 32 F2 families were recombinant whereas each of the remaining recurrent parents varied from 0-2 recombinant phenotypes per 32 F2 families. There is nothing unusual in the pedigree of Hardin (Fehr et al., 1983) which would suggest that recombination values would be affected.

The cytoplasmic groupings, based upon mitochondrial and chloroplastic DNA, for the 34 lines were not complete. Data for only 16 lines were available, and 14 lines, including Hardin, had the Bedford-type cytoplasm (Grabau et al., 1989, 1992; Hanlon and Grabau, 1995), based on mitochondrial DNA determinations. The same 16 lines had the same chloroplastic DNA grouping (Close et al., 1989; Lee et al., 1994).

Variation in recombination values have been noted in soybean (Pfeiffer and Vogt, 1990; Pfeiffer, 1993; Griffin and Palmer, 1987; Palmer and Chen, 1998). Even though the recombination values were statistically different between the *ms6-w1* donor cytoplasm and the recurrent parent cytoplasms in the F2-family test, the differences were small. These differences will not impede using the cosegregation method (Lewers et al., 1996) to produce hybrid soybean seed.

ACKNOWLEDGMENTS

We gratefully acknowledge seed from Asgrow Seed Co., CIBA Seeds, DeKalb Genetics Corp., Jacques Seed Co., Land O'Lakes, Inc., Northrup King Co., Pioneer Hi-Bred, Int'l., and ProfiSeed, Inc.

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